

## ORIGINAL

# Wakame (*Undaria pinnatifida*) modulates hyperphosphatemia in a rat model of chronic renal failure

Kanako Katai, Aya Iwamoto, Yuka Kimura, Yuki Oshima, Saori Arioka, Yuki Morimi, Ayaka Omuro, and Teruko Nakasa

Department of Food science and Nutrition, Faculty of Human Life and Science, Doshisha Women's College of Liberal Arts, Japan

**Abstract :** In chronic renal failure, inorganic phosphate (Pi) retention speeds up the progression to end-stage renal disease. The current therapy for hyperphosphatemia in patients with chronic renal failure consists of dietary Pi restriction combined with administration of Pi binders, but each therapy has practical problems. Thus, the discovery of foods or nutrients that inhibit Pi absorption may be useful for the treatment of hyperphosphatemia. In the present study, we investigated whether wakame (*Undaria pinnatifida*) is a useful food for the prevention of hyperphosphatemia in a rat model of renal failure. Feeding a diet containing 5% wakame significantly decreased plasma and urinary Pi levels and increased the amount of fecal Pi. In addition, wakame significantly reduced plasma blood urea nitrogen and plasma Pi levels in 5/6 nephrectomized rats fed a high-Pi diet. Biochemical analyses showed that the reduction of intestinal Pi absorption is the main reason for the decrease in plasma Pi levels in rats fed a diet containing wakame. In addition, feeding alginic acid and fucoidan, major components of wakame fiber, was effective in reducing plasma Pi levels in normal rats. Finally, we concluded that wakame may be a useful food for the prevention of hyperphosphatemia in rodents. *J. Med. Invest.* 62 : 68-74, February, 2015

**Keywords :** inorganic phosphate, hyperphosphatemia, seaweed, fucoidan, alginic acid

## INTRODUCTION

Chronic kidney disease (CKD) is associated with abnormal bone and mineral metabolism, resulting in systemic vascular calcification. Phosphorus retention is a major harmful complication of CKD that leads to ectopic calcification and significant risk of cardiovascular morbidity and mortality (1-5). Secondary hyperparathyroidism, induced by hyperphosphatemia and 1,25-(OH)<sub>2</sub> vitamin D<sub>3</sub> deficiency, is accompanied by parathyroid hyperplasia and excessive synthesis and secretion of parathyroid hormone, which results in renal osteodystrophy (4-6). These pathologies have established the concept of CKD-mineral and bone disorder (CKD-MBD), which recognizes that CKD leads to higher morbidity and mortality due to cardiovascular disease and poor life prognosis (1, 2, 4). In response, a treatment strategy that emphasizes life prognosis has been recommended throughout the world. It is considered important to control the blood concentrations of phosphorus and calcium within the normal range in end-stage CKD patients (1-3).

Hyperphosphatemia is treated with drug therapy as well as with diet therapy. The drugs used in this context are adsorbents that absorb dietary phosphorus in the digestive tract and excrete it in the feces (7, 8). However, each of these drugs has side effects that require diet therapy with a phosphorus-restricted diet to ensure the safest and most reliable treatment (9, 10). In the present study, we focused on wakame (*Undaria pinnatifida*), which has a variety of biological functions in health and disease.

Wakame (*U. pinnatifida*), which is traditionally eaten more often in Japan than in the rest of the world (11), is useful as a source of minerals and dietary fiber (12).

The dietary fiber found in seaweed species amounts to 30% of dry weight and includes alginic acid and fucoidan (AF), which have a

chemical structure different from that of the dietary fiber found in vegetables and grains (12). Alginic acid, a viscous polysaccharide, is a viscous component of seaweed that can be obtained from brown algae as well as from wakame (13). Fucoidan is the generic name for polysaccharides that contain a monosaccharide called L-fucose as their principal component (14, 15). Fucoidan also contains D-glucuronic acid, D-galactose, D-mannose, and sulfate groups in constituent sugars (16, 17). Fucoidan extracted from wakame has anti-inflammatory (18), immunomodulatory (19), antitumor (20), and anticoagulation activities (21) and organ protective effects that include protection of the digestive tract (17). In the present study, we investigated the effect of wakame on the control of plasma levels of inorganic phosphate (Pi) in normal rats and rats experiencing chronic renal failure.

## MATERIALS AND METHODS

### Materials

Wakame (brand name is Hamamidori), alginic acid, and fucoidan (fucoidan isolated from Mekabu (thick wakame leaves) were obtained from the Riken Vitamin Co., Ltd. (Tokyo, Japan).

### Animals

Normal Wistar rats and 5/6 nephrectomized (5/6NX) rats were purchased from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan). The (5/6NX) rats were established by general method (22). The 5/6 nephrectomy was performed at the age of 7 weeks under anesthesia with sodium pentobarbital (50 mg/kg body weight, i.p.). The operation began with ablation of approximately the entire left kidney, and then 2/3 of the right kidney was removed by ligation of the renal artery, vein, and ureter 1 week later. After acclimatization (2 weeks), the 5/6NX rats were divided into two groups by the follow-up period. Rats were maintained under pathogen-free conditions and handled in accordance with the Guidelines for Animal Experimentation of Doshisha Women's College of Liberal Arts.

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Address correspondence and reprint requests to Kanako Katai, Department of Food science and Nutrition, Faculty of Human Life and Science, Doshisha Women's college of Liberal Arts, Teramachi Nishi-iru, Imadegawa-dori, Kamigyo-ku, Kyoto 602-0893, Japan and Fax : +81-75-251-4235.

### Animal Diets

Rats were fed AIN93-G, a standard diet for growth period, pregnant period, and lactating period, obtained from Oriental Yeast Co., Ltd (Tokyo, Japan), which was partially modified (Table 1). The basic composition of AIN93-G includes a phosphorus concentration of 0.3% and a calcium concentration of 0.5%. To modify AIN93-G to achieve a high-phosphorus diet, AIN93-G was supplemented with  $\text{KH}_2\text{PO}_4$  (23, 24) and polyphosphoric acid in amounts adjusted to achieve a final phosphorus concentration of 1.2% (Table 1). Dried wakame was crushed into powder and then mixed with various diets. The amount of wakame added to each feed was set at 5% (25, 26). The amount of added arginic acid and fucoidan, converted to the amount contained in wakame, was set at 1.75% arginic acid and 0.5% fucoidan (Tables 1 and 2). Corrections associated with the addition of feed components were done with cornstarch. Following preliminary feeding, rats were divided into two groups. The control Pi (CP) group was fed AIN93-G (0.3% Pi; Oriental Yeast Co., Ltd, Tokyo, Japan) for 3 weeks, and the high-Pi (HP) group was fed AIN93-G modified to contain 1.2% Pi for 3 weeks. Subsequently, both groups were further divided into two groups so as to avoid any intergroup differences in body weight. The wakame group (CP+W or HP+W groups) had their diets supplemented with 5% wakame, whereas the rats in the other group were fed their original (CP or HP) diets. Rats were sacrificed at 16 weeks of age.

Table 1. Composition of the experimental diets

	CP	CP+W	HP	HP+W	HP+AF
Cornstarch (g)	39.7486	34.7486	36.0620	31.0620	33.8120
Casein (g)	20.0000	20.0000	20.0000	20.0000	20.0000
$\alpha$ -cornstarch (g)	13.2000	13.2000	13.2000	13.2000	13.2000
Sucrose (g)	10.0000	10.0000	10.0000	10.0000	10.0000
Soy oil (g)	7.0000	7.0000	7.0000	7.0000	7.0000
Cellulose (g)	5.0000	5.0000	5.0000	5.0000	5.0000
Mineral Mix <sup>(1)</sup> (g)	3.5000	3.5000	3.5000	3.5000	3.5000
Vitamin Mix <sup>(2)</sup> (g)	1.0000	1.0000	1.0000	1.0000	1.0000
L-Cystine (g)	0.3000	0.3000	0.3000	0.3000	0.3000
Choline bitartrate (g)	0.2500	0.2500	0.2500	0.2500	0.2500
Tertiary butylhydroquinone (g)	0.0014	0.0014	0.0014	0.0014	0.0014
$\text{KH}_2\text{PO}_4$ (g)	0.0000	0.0000	3.0755	3.0755	3.0755
$\text{P}_2\text{O}_5$ (g)	0.0000	0.0000	0.6111	0.6111	0.6111
Wakame (g)	0.0000	5.0000	0.0000	5.0000	0.0000
Arginic acid (g)	0.0000	0.0000	0.0000	0.0000	1.7500
Fucoidan (g)	0.0000	0.0000	0.0000	0.0000	0.5000
Total (g)	100.0000	100.0000	100.0000	100.0000	100.0000

(1) AIN-93G Mineral Mixture (2) AIN-93G Vitamin Mixture

Table 2. Comparison of the minerals in experimental diets

	CP	CP+W	HP	HP+W	HP+AF
Na (%)	0.103	0.433	0.103	0.433	0.103
K (%)	0.334	0.594	1.218	1.478	1.218
Ca (%)	0.5	0.539	0.5	0.539	0.5
Mg (%)	0.051	0.106	0.051	0.106	0.051
Pi (%)	0.3	0.318	1.2	1.218	1.2

### Biochemical analysis of blood, urine, and feces

Phosphorus concentrations in plasma, urine, and feces were measured with the Phospho C test Kit (Wako Pure Chemical Industries, Ltd) using the *p*-methylaminophenol reduction method. Calcium concentrations in plasma, urine, and feces were measured with the Calcium E Test Kit (Wako Pure Chemical Industries, Ltd) using the MXB method. Plasma and urine creatinine concentrations were measured with the Lab Assay Creatinine Test Kit (Wako Pure Chemical Industries, Ltd) using the Jaffe method. Blood urea nitrogen (BUN) concentrations were measured with the Blood Nitrogen B Test Kit (Wako Pure Chemical Industries, Ltd) using the urease-indophenol method.

### Statistical analyses

Data are expressed as means and standard deviations or means and their standard errors. Statistical analyses were performed using ANOVA. Multiple comparisons (Tukey's test) were conducted in cases of significant difference. Student's *t*-test was used for comparisons between two groups.

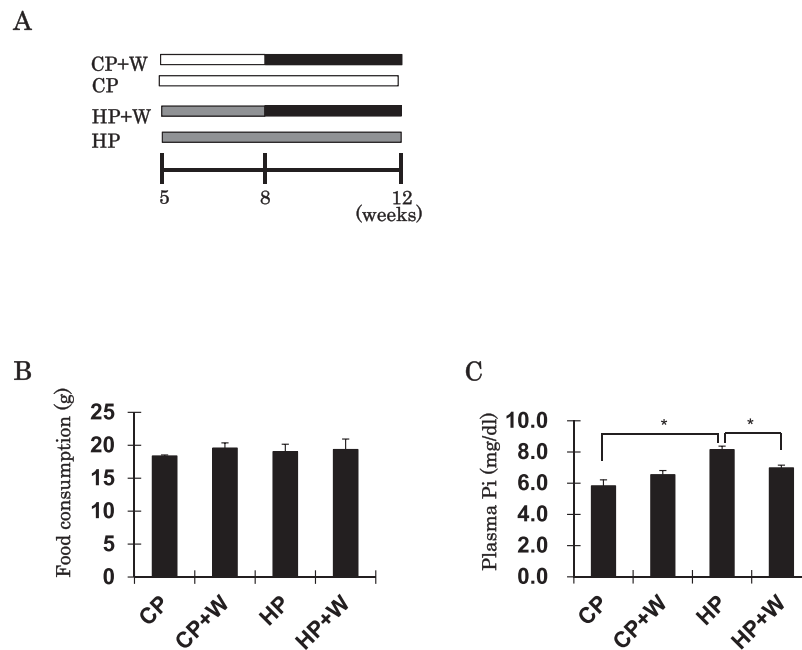
## RESULTS

### Effect of wakame on plasma Pi levels in normal rats

In the first set of experiments (Figure 1A), we investigated the effect of wakame on plasma Pi levels in normal rats. The compositions of the experimental diets are listed in Tables 1 and 2. The experimental animals were fed either a HP diet (1.2% Pi; HP group) or a CP diet (0.3% Pi; CP group) for 3 weeks. Each group was then split into two; half were fed a diet containing wakame (W), and the other half were continued on their CP or HP diets for 4 weeks. As shown in Figure 1B, food intake did not differ between the HP and CP groups. Plasma Pi levels were significantly higher in the HP group, compared with the CP group. The final concentration of dietary Pi was slightly increased in the CP+W (0.318% Pi) diet, compared with the CP diet (0.3% Pi). The concentrations of minerals (Na, K, Ca, Mg, and Pi [%]) in the various diets are listed in Table 2. The diet containing wakame contained higher levels of Na, K, and Mg than the control diet. However, the increased levels of these minerals in the diet did not affect the plasma Pi concentration in normal rats, because a control diet containing higher Na, K, and Mg levels (wakame mineral mixture) did not influenced plasma Pi levels in normal rats (data not shown). Indeed, the CP and the CP+W groups showed normal plasma Pi levels (Figure 1C). In contrast, the animals fed a HP diet for 7 weeks showed significantly higher plasma Pi levels than those fed a normal Pi diet. In the animals fed a HP diet containing wakame (the HP+W group), plasma Pi levels were significantly reduced compared with the HP group (Figure 1C). These data suggest that the diet containing wakame prevented the hyperphosphatemia induced by a HP diet.

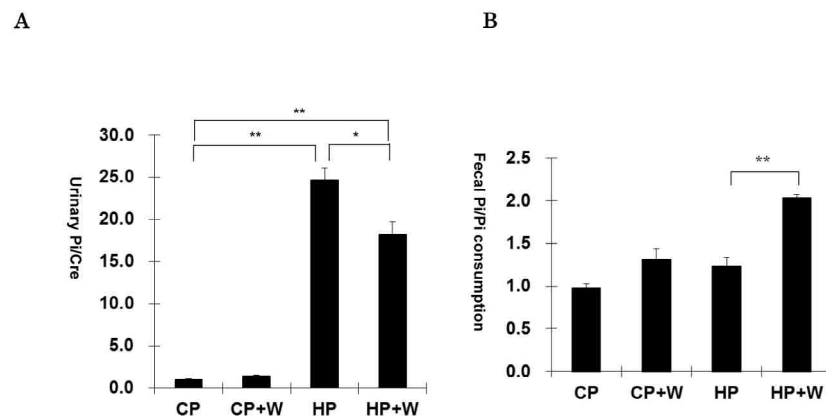
### Effect of wakame on urinary and fecal Pi levels in normal rats

To address the mechanisms of wakame's plasma Pi lowering effect, we analyzed urinary and fecal Pi levels. If wakame prevents the intestinal absorption of Pi, the experimental animals should show a reduction in urinary Pi levels and an increase in fecal Pi levels. Urinary Pi excretion levels were significantly increased in the HP group, compared with those in the CP group (Figure 2A). This occurred because feeding a diet containing high amounts of Pi stimulates urinary Pi excretion. Comparing the CP+W group with the CP group, urinary Pi excretion levels were not changed (Figure 2A). In contrast, urinary Pi excretion levels were significantly decreased in the HP+W group, compared with the HP group (Figure 2A). In the HP+W group, fecal Pi levels were significantly increased to about 1.65-fold that of the HP group (Figure 2B).



**Figure 1.** Plasma Pi levels are decreased by wakame in normal rats fed a high-Pi diet.

(A) A schematic of the experimental design that defines the various diet groups. CP : Control Pi (AIN93-G) diet used to establish normative parameters. CP+W : CP diet supplemented with 5% wakame. HP : AIN93-G diet supplemented to contain 1.2% Pi. HP+W : HP diet further supplemented with 5% wakame. CP and HP groups were fed CP or HP diet for 3 weeks. Subsequently, both groups were further divided into two groups (including 5% wakame or not) so as to avoid any intergroup differences in body weight at 8 weeks of age. Rats were fed this diet for the next 4 weeks. (B) Food consumption in the various diet groups measured at 12 weeks of age. (C) Plasma Pi levels analyzed at 12 weeks of age. Data are presented as mean  $\pm$  SEM ( $n=6$ /group). \* $P<0.05$



**Figure 2.** Effect of wakame on urinary and fecal Pi levels.

(A) The experimental design followed that shown in Figure 1A. Urinary Pi/urinary creatinine (cre). (B) Fecal Pi/Pi consumption was analyzed at 12 weeks of age. Data are presented as mean  $\pm$  SEM ( $n=6$ /group). \* $P<0.05$ ; \*\* $P<0.01$ .

These data show that intestinal absorption of Pi was significantly decreased in the HP+W group, compared with the HP group. This suggests that the reduced absorption of intestinal Pi is a primary mechanism for the decrease in plasma Pi levels seen in the animals fed a diet containing wakame.

#### *Effect of wakame components on the progression of CKD*

Next to investigate the effect of wakame on the progression of renal failure, we used 5/6NX animals fed a HP diet (22) (Figure 3A). In this model, feeding a HP diet to 5/6NX rats accelerates renal damage. We compared the biochemical parameters of the HP with those of the HP+W group. Food intake did not differ between the HP+W and HP groups (Figure 3B). Plasma Pi levels

were significantly decreased in the HP+W group, compared with the HP group (Figure 3C). Although urinary Pi excretion levels were the same in the HP+W and HP groups (Figure 3D), fecal Pi levels were increased about 1.55-fold in the HP+W group, compared with the HP group (Figure 3E). The BUN levels, a parameter of kidney function, were significantly reduced in the HP+W group (Figure 3F). These data show that, in a model of CKD, feeding a diet containing wakame inhibits the progression of renal failure and hyperphosphatemia.

#### *Effect of wakame fiber components, AF, on plasma Pi levels*

We performed a search to identify the components of wakame that lower the plasma Pi level. Because fiber accounts for 30% of

the total components of dry wakame, we prepared diets containing levels of AF that are the same as those found in the diets containing wakame. The amounts of alginic acid (A) and fucoidan (F) in each diet containing HP are listed in Table 1. We then investigated whether the fibers found in wakame, AF, reduce plasma Pi levels

in the CP and the HP groups (Figure 4). In the CP+AF and the HP+AF groups, plasma Pi levels were significantly reduced compared with those in the CP and the HP groups (Figure 4A and B). These data suggest that AF reduce plasma Pi levels in normal rats.

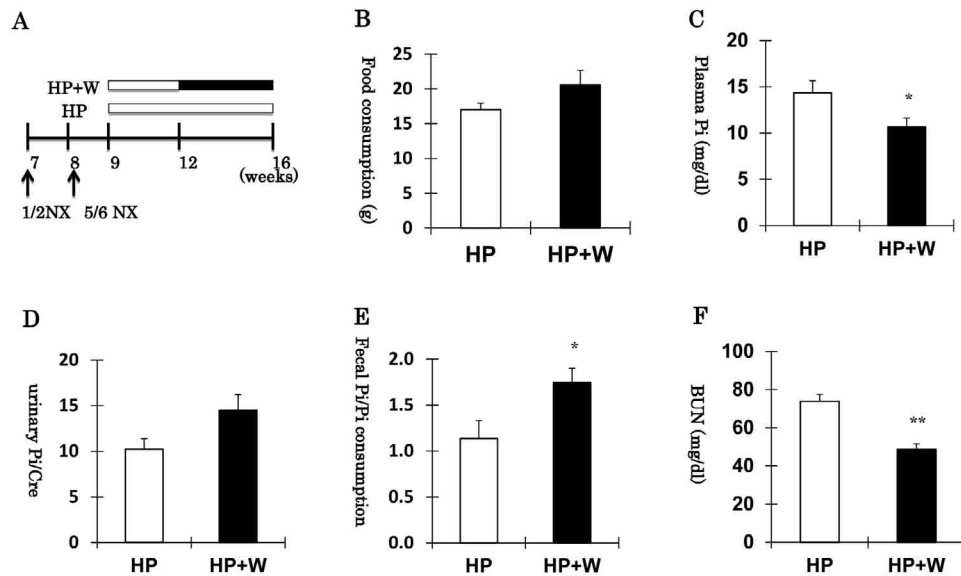


Figure 3. Effect of wakame on plasma Pi levels on 5/6 nephrectomized rats.

(A) A schematic of the experimental design defining the various diet groups. HP: AIN93-G diet supplemented to contain 1.2% Pi. HP+W: HP further supplemented to contain 5% wakame. (B) Food consumption by rats of each diet. (C) Plasma Pi levels. (D) Urinary Pi/creatinine (cre). (E) Fecal Pi/Pi consumption. (F) Serum BUN levels. These parameters were analyzed at 16 weeks of age. Data are presented as mean  $\pm$  SEM. ( $n=6$ /group). \*\* $P < 0.05$ ; \*\* $P < 0.01$ .

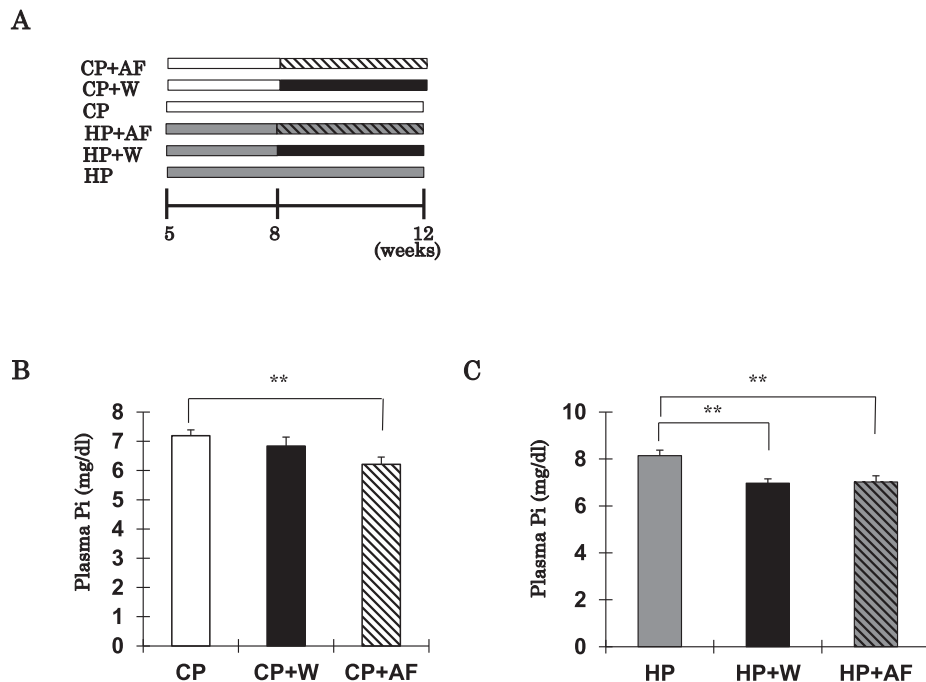


Figure 4. Effect of alginic acid and fucoidan as wakame fiber on plasma Pi levels in normal rats.

(A) A schematic of the experimental design defining the various diet groups. CP: Control Pi (AIN93-G) diet used to establish normative parameters. CP+W: CP diet supplemented with 5% wakame. CP+AF: CP diet supplemented with alginic acid (1.75%) and fucoidan (0.5%). HP: AIN93-G diet supplemented to include 1.2% Pi. HP+W: HP diet further supplemented with 5% wakame. HP+AF: HP diet further supplemented with 1.75% alginic acid and 0.5% fucoidan. (B) and (C) Plasma Pi levels. Data are presented as mean  $\pm$  SEM. \*\* $P < 0.01$  ( $n=4-6$ /group).

## DISCUSSION

Hyperphosphatemia has been identified in the past decade as a strong predictor of mortality in patients with advanced CKD (4, 27, 28). More recent studies have shown that the association between HP concentrations and higher mortality is not restricted to persons with renal disease; it can also be observed in persons with cardiovascular disease and even in the general population (29). Current strategies for the treatment of hyperphosphatemia in dialysis patients include dietary phosphate restriction and oral phosphate binders, although these treatments, if used aggressively, can lead to malnutrition, adverse gastrointestinal effects, and poor compliance with all medications, particularly in the elderly (7). As an alternative, a number of studies have shown that bioactive compounds extracted from natural products have some therapeutic potential for hyperphosphatemia. We previously reported that nicotinamide (an amide derivative of the water-soluble vitamin B3) is a potentially interesting alternative to phosphate binders (30, 31). We demonstrated that nicotinamide reduces hyperphosphatemia by inhibiting a sodium-dependent phosphate co-transporter in the kidney and small intestine (30, 31). Therefore, discovering foods or nutrients that inhibit Pi absorption may be useful for the treatment of hyperphosphatemia.

In the present study, we investigated the plasma Pi lowering effects of wakame, as well as wakame's effect on the progression of renal failure. Wakame (*U. pinnatifida*) is a food that is traditionally eaten more often in Japan than in the rest of the world. The fiber in seaweed species amounts to 30% of dry weight and includes AF, which have chemical structures different from those of the dietary fibers found in vegetables and grains. In recent toxicological reports, fucoidan derived from *U. pinnatifid* and *Laminaria japonica* was found to be safe in animal models at very high levels of intake (32, 33). In addition, wakame is a useful source of sodium, potassium, and magnesium (34). These factors may not be involved in the prevention of hyperphosphatemia, because the elevation of total mineral content in the experimental diets did not affect plasma Pi levels (data not shown). Therefore, we hypothesized that dietary fiber may be involved in the prevention of hyperphosphatemia in animals with CKD.

In normal animals, diets containing 5% wakame significantly decreased plasma Pi levels and urinary Pi excretion. The diet containing 5% wakame also prevented the progression of renal failure. Animals fed the diet containing wakame showed a significant increase in fecal Pi excretion. We suggest that this effect may be due to a reduction in plasma Pi levels or uremic toxins. Indeed, wakame has many functional elements for toxin binding (35). In the present study, wakame lowered plasma Pi levels by preventing the absorption of intestinal Pi and prevented the progression of renal failure. Wakame may be useful food for the prevention of hyperphosphatemia.

Alginic acid is a viscous component of seaweed that can be obtained from brown algae as well as from wakame. It is a polysaccharide with an  $\alpha$ ,  $\beta$ 1-4 coupling involving D-mannuronic acid and L-glucuronic acid (13). Alginic acid is famous for its metal chelating ability and is used widely as a healthy food because of its beneficial functions (13). Studies of the effect of alginic acid on mineral absorption have been reported. Hodgkinson *et al.* reported its interference with calcium absorption in humans (36). Alginic acid also increases fecal losses of copper, iron, and zinc in rats (37, 38). In addition, low molecular weight sodium alginate inhibits reabsorption of bile acid by binding to bile acid in the small intestine and causing its excretion in the feces (39).

The study of this process has shown that bile acid is synthesized from the cholesterol to supplement the bile acid lost because of inhibited reabsorption, and, as a result, the plasma cholesterol level is lowered (39, 40).

Fucoidan is the generic name for polysaccharides containing the monosaccharide L-fucose as their principle component. Fucoidan also contains D-glucuronic acid, D-galactose, D-mannose, and sulfate groups in constituent sugars (17). Recent in vivo and in vitro clinical research shows that fucoidan is useful to control acute and chronic inflammation via selectin blockade, enzyme inhibition, and inhibition of the complement cascade (41). Fucoidan is also known to increase the activity of fibroblast growth factor by binding and to have an anticancer effect (42, 43). Although we have not analyzed the mechanisms of alginic acids and fucoidan on lowering of plasma Pi levels, there are several possibilities on the lowering effect of plasma Pi by both factors.

Recent studies indicate that the intestinal sodium-dependent phosphate co-transporters Npt2b are a major target for the prevention of hyperphosphatemia in animal models of CKD (44-46). Indeed, Npt2b knockout mice show resistance to hyperphosphatemia in CKD models (45). The N-glycan of the Npt2 family transporters is important in their function and cellular localization. Recent studies showed that klotho modifies the N-glycan of Npt2a/b, thereby inhibiting Na-dependent Pi co-transport activity (47). Klotho hydrolyzes  $\beta$ -glycosides and is a  $\beta$ -D-glucuronidase inhibitor (48). Chang *et al.* proposed that klotho activates TRPV5 via its  $\beta$ -glucuronidase activity (48). The Mekabu fucoidan commonly used contains a high proportion of galactose (17). Therefore, these components may be involved in the modification of activities mediated by the N-glycan of Npt2b in the small intestine (data not shown). This study is the first report that Wakame has ameliorating effect for hyperphosphatemia in rats experiencing renal failure. Further studies are needed to clarify the lowering effect of AF on plasma Pi levels in CKD models. Finally, in the present study, we showed that wakame is a useful food for the prevention of hyperphosphatemia in an animal model of CKD.

## ABBREVIATIONS

Pi inorganic phosphate  
CKD chronic kidney disease  
CKD-MBD CKD-mineral and bone disorder  
AF alginic acid (A) and fucoidan (F)  
5/6NX 5/6 nephrectomized  
CP control phosphate  
CP+W control phosphate+wakame  
HP high phosphate  
HP+W high phosphate+wakame

## CONFLICT OF INTEREST

No potential conflicts of interest were disclosed.

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